NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 437

TOXICOLOGY AND CARCINOGENESIS STUDIES OF HEXACHLOROCYCLOPENTADIENE

(CAS NO. 77-47-4)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF HEXACHLOROCYCLOPENTADIENE

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NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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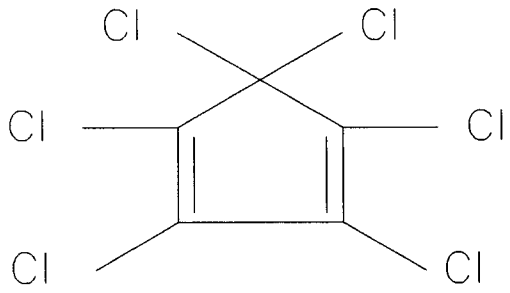
ABSTRACT



HEXACHLOROCYCLOPENTADIENE

CAS No. 77-47-4

Chemical Formula: C_5Cl_6 Molecular Weight: 272.8

Synonyms: Perchlorocyclopentadiene, hexachloro-1,3-cyclopentadiene, HEX, HCPD, HCCP, HCCPD
Trade Name: C-56-Graphlox

Hexachlorocyclopentadiene is an intermediate used in the manufacture of flame retardants, resins, and chlorinated cyclodiene pesticides. Toxicology and carcinogenesis studies were conducted by exposing male and female F344/N rats and B6C3F$_1$ mice to atmospheres containing hexachlorocyclopentadiene (approximately 98% pure) for 6 hours per day, 5 days per week, for 13 weeks or 2 years. A stop-exposure evaluation was conducted in male B6C3F$_1$ mice to determine the influence of exposure level and exposure duration on the development of nonneoplastic lesions of the respiratory tract and on their regression or progression after exposure was stopped. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, *Drosophila melanogaster*, and mouse peripheral blood samples were analyzed for frequency of micronucleated normochromatic erythrocytes.

13-Week Study in Rats

Groups of 10 male and 10 female rats were exposed to atmospheres containing 0, 0.04, 0.15, 0.4, 1, or 2 ppm (equivalent to 0, 0.45, 1.67, 4.46, 11.14, and 22.28 mg/m^3) hexachlorocyclopentadiene. Additional rats were exposed to 0, 0.04, 0.4, or 2 ppm hexachlorocyclopentadiene and evaluated for differences in clinical pathology parameters. All rats in the 1 and 2 ppm groups died during the first 4 weeks of the study. The final mean body weight and mean body weight gain of males exposed to 0.4 ppm were significantly lower than those of the controls. Listlessness was observed in 2 ppm rats from week 1, in 1 ppm rats from week 2, and in 0.4 ppm rats during week 3. Rats exposed to 1 or 2 ppm also experienced respiratory distress. No chemical-related differences in hematology, clinical chemistry, or urinalysis parameters were observed in male or female rats. Absolute and relative lung weights of 0.4 ppm males were significantly greater than those of the controls. Inflammation (necrotizing, chronic, or suppurative) of the nose, larynx, trachea, and lung was observed in 0.4, 1, and 2 ppm males and females. Squamous metaplasia of the epithelial lining of the nose of 0.4 ppm males and 1 and 2 ppm males and females was also observed.

13-Week Study in Mice

Groups of 10 male and 10 female mice were exposed to atmospheres containing 0, 0.04, 0.15, 0.4, 1, or 2 ppm (equivalent to 0, 0.45, 1.67, 4.46, 11.14, and

22.28 mg/m^3) hexachlorocyclopentadiene. Additional mice were exposed to 0, 0.04, 0.4, or 2 ppm and evaluated for differences in clinical pathology parameters. All 2 ppm mice died during the first week of exposure. All 1 ppm mice died during the first 5 weeks of exposure. Five males and two females in the 0.4 ppm group died during the first 2 weeks of exposure. Deaths in the other groups were not related to hexachlorocyclopentadiene exposure. Final mean body weights of males exposed to 0.15 and 0.4 ppm and the body weight gain of 0.4 ppm males were significantly lower than those of the controls. Treatment-related clinical findings included listlessness in 0.4 and 1 ppm males and females. No chemical-related differences in hematology, clinical chemistry, or urinalysis parameters were observed in male or female mice. Necrosis or inflammation of the nose, larynx, trachea, or lung occurred in mice exposed to 0.4, 1, and 2 ppm hexachlorocyclopentadiene. Squamous metaplasia of the larynx or trachea was observed in 0.15, 0.4, and 1 ppm males and in 0.4 and 1 ppm females.

2-YEAR STUDY IN RATS

Survival, Body Weights, Clinical Findings, and Urinalysis

Groups of 60 male and 60 female rats were exposed to atmospheres containing 0, 0.01, 0.05, or 0.2 ppm (equivalent to 0, 0.11, 0.56, and 2.28 mg/m^3) hexachlorocyclopentadiene. Survival rates and mean body weights of exposed rats were similar to those of the controls. No chemical-related clinical findings were observed in male or female rats during the 2-year study. No differences in urinalysis parameters at the 15-month interim evaluation could be attributed to exposure to hexachlorocyclopentadiene.

Pathology Findings

No increases in neoplasm incidences could be attributed to hexachlorocyclopentadiene. Toxicity was limited to the respiratory tract and included an increase in the incidence of pigmentation of the respiratory epithelium of the nose, trachea, and the bronchi and bronchioles of the lung in both males and females. Exposure to hexachlorocyclopentadiene also caused an increase in the incidence of squamous metaplasia of the laryngeal epithelium of exposed females; the incidences in 0.01 and 0.2 ppm females were significantly greater than that of the controls. The severity of squamous metaplasia was minimal in all exposed and control females.

2-YEAR STUDY IN MICE

Survival, Body Weights, Clinical Findings, and Urinalysis

Groups of 60 male and 60 female mice were exposed to atmospheres containing 0, 0.01, 0.05, or 0.2 ppm (equivalent to 0, 0.11, 0.56, and 2.28 mg/m^3) hexachlorocyclopentadiene. The 2-year survival rate of female mice in the 0.2 ppm group was marginally lower than that of the controls due to a higher incidence of ovarian inflammation in 0.2 ppm females. Mean body weights of 0.2 ppm males (weeks 62 to 103) and females (throughout the study) were lower than those of the controls. No clinical findings in male or female mice were attributed to chemical exposure during the 2-year study. There were no chemical-related differences in urinalysis parameters at the 15-month interim evaluation.

Pathology Findings

The site of toxicity of hexachlorocyclopentadiene exposure in mice in the 2-year study was the respiratory tract. Chemical-related pigmentation of the respiratory epithelium of the nose, trachea, and lung and suppurative inflammation of the nose were observed. No increased neoplasm incidences in males or females could be attributed to hexachlorocyclopentadiene exposure.

STOP-EXPOSURE EVALUATION

Survival, Body Weights, and Clinical Findings

Groups of male mice were exposed to atmospheres containing 0.2 ppm hexachlorocyclopentadiene for 33 or 66 weeks or 0.5 ppm for 26 or 42 weeks followed by exposure to air until the end of the study. Fifty male mice from each stop-exposure group were evaluated at 2 years. Two-year survival rates of stop-exposure groups were similar to that of the controls. Final mean body weights of stop-exposure groups were similar to that of the controls. No chemical-related clinical findings were observed.

Pathology Findings

Nonneoplastic respiratory tract lesions similar to those observed in the core study were observed in males in the stop-exposure groups. Chemical-related pigmentation and inflammation of the respiratory epithelium were persistent as indicated by their presence in many male mice after recovery periods of 62 to 78 weeks, and the incidence and severity of the lesions were related to exposure concentration and duration.

GENETIC TOXICOLOGY

Hexachlorocyclopentadiene was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 when tested with and without S9. Hexachlorocyclopentadiene did induce sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, with and without S9. No induction of sex-linked recessive lethal mutations was observed in male *Drosophila melanogaster* treated with hexachlorocyclopentadiene by feeding or injection, and no increase in the frequency of micronucleated erythrocytes was seen in male or female B6C3F$_1$ mice exposed to hexachlorocyclopentadiene by inhalation for 13 weeks.

CONCLUSIONS

Under the conditions of these 2-year studies, there was *no evidence of carcinogenic activity** of hexachlorocyclopentadiene in male or female F344/N rats or B6C3F$_1$ mice exposed to 0.01, 0.05, or 0.2 ppm.

Exposure of rats to hexachlorocyclopentadiene produced pigmentation of the respiratory epithelium of the nose, trachea (males), and bronchi and bronchioles of the lung. Squamous metaplasia of the laryngeal epithelium occurred in female rats exposed to hexachlorocyclopentadiene. Suppurative inflammation of the nose as well as pigmentation of the respiratory mucosal epithelium occurred in mice exposed to hexachlorocyclopentadiene.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Hexachlorocyclopentadiene

Variable	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 0.01, 0.05, or 0.2 ppm by inhalation (equivalent to 0, 0.11, 0.56, or 2.28 mg/m^3)	0, 0.01, 0.05, or 0.2 ppm by inhalation (equivalent to 0, 0.11, 0.56, or 2.28 mg/m^3)	0, 0.01, 0.05, or 0.2 ppm by inhalation (equivalent to 0, 0.11, 0.56, or 2.28 mg/m^3)	0, 0.01, 0.05, or 0.2 ppm by inhalation (equivalent to 0, 0.11, 0.56, or 2.28 mg/m^3)
Body weights	Exposed groups similar to controls	Exposed groups similar to controls	High dose lower than controls	High dose lower than controls
2-Year survival rates	36/50, 33/50, 45/50, 32/50	28/50, 33/50, 30/49, 30/50	35/50, 33/50, 42/50, 34/50	31/50, 32/50, 30/50, 21/50
Nonneoplastic effects	Lung: bronchiole pigmentation (0/50, 0/50, 0/50, 49/50); peribronchiolar pigmentation (0/50, 0/50, 2/50, 16/50) Nose: pigmentation (1/48, 46/50, 48/49, 48/50) Trachea: pigmentation (0/48, 0/50, 0/48, 5/50)	Larynx: squamous metaplasia (9/50, 20/50, 15/48, 24/50) Lung: bronchiole pigmentation (0/50, 25/50, 42/49, 50/50); peribronchiolar pigmentation (3/50, 1/50, 4/49, 27/50) Nose: pigmentation (0/50, 34/50, 47/49, 48/50)	Lung: mucosal pigmentation (0/49, 2/50, 42/50, 45/50) Nose: suppurative inflammation (0/50, 0/50, 1/50, 36/50); mucosal pigmentation (0/50, 45/50, 50/50, 44/50) Trachea: mucosal pigmentation (0/50, 29/50, 48/50, 48/50)	Lung: mucosal pigmentation (0/48, 0/50, 27/50, 44/49) Nose: suppurative inflammation (4/49, 0/50, 3/50, 40/48); mucosal pigmentation (0/49, 40/50, 48/50, 41/48) Trachea: mucosal pigmentation (0/49, 6/50, 43/48, 42/47)
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence

Genetic toxicology

Salmonella typhimurium gene mutation:	Negative with and without S9 in strains TA98, TA100, TA1535, and TA1537
Sister chromatid exchanges	
Chinese hamster ovary cells *in vitro*:	Positive with and without S9
Chromosomal aberrations	
Chinese hamster ovary cells *in vitro*:	Positive with and without S9
Sex-linked recessive lethal mutation in *Drosophila melanogaster*:	Negative administered in feed or by injection
Mouse peripheral blood erythrocytes *in vivo*:	Negative at 13 weeks

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such neoplasms to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant neoplasm incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in neoplasm induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed neoplasm increase;
- concurrent control neoplasm incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on hexachlorocyclopentadiene on 22 June 1993 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 22 June 1993 the draft Technical Report on the toxicology and carcinogenesis studies of hexachlorocyclopentadiene received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of hexachlorocyclopentadiene by discussing the uses of the chemical, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related nonneoplastic lesions in rats and mice. He said a stop-exposure evaluation in male mice was done to determine whether there was regression or progression of metaplastic lesions in the respiratory tract. The proposed conclusions were *no evidence of carcinogenic activity* in male or female F344/N rats or male or female B6C3F$_1$ mice.

Dr. Zeise, a principal reviewer, agreed in principle with the proposed conclusions. She thought that rats may have been able to tolerate higher doses, as indicated by the survival, mean body weights, and clinical findings in the 2-year study, and that this should be noted in the abstract and elsewhere. Dr. Zeise said that there needed to be more discussion of the significance of the alveolar epithelial hyperplasia seen in male mice in the stop-exposure evaluation. Dr. Abdo agreed.

Dr. Ward, the second principal reviewer, also agreed in principle with the proposed conclusions and stated that rats might have been able to tolerate a higher top dose because no effects on body weight gain or survival were observed and because toxic lesions were limited to pigmentation of the respiratory tract epithelium and mild squamous metaplasia in the larynx of females. Dr. Abdo responded that the sharp increase in mortality between rats exposed to 0.4 and 1.0 ppm along with the decreased body weight gain of 0.4 ppm males in the 13-week study justified the top dose chosen for the 2-year study. Dr. Ward criticized the use of less than 50 animals for complete histopathology in the 0.01 and 0.05 ppm groups, and wondered if the reduced statistical power might have affected interpretation in organs where there were equivocal effects. Dr. S.L. Eustis, NIEHS, noted that the NTP has used the reduced protocol for many years, and that the only case in this study where use of a full protocol might have resolved uncertainty was pituitary gland neoplasms in male rats.

Dr. Davidson, the third principal reviewer, agreed with the proposed conclusions. She said information should be added to the abstract to describe the severity of the respiratory lesions and to explain how the exposure concentrations and durations were selected for the stop-exposure evaluation.

Mr. Beliczky asked that the report include comment on eye examinations and effects. Dr. G.N. Rao, NIEHS, responded that rodents close their eyes when exposed to an irritant chemical and that this might explain why no ocular lesions were observed. Dr. van Zwieten observed that there were significantly increased incidences of squamous metaplasia of the larynx in 0.01 and 0.2 ppm females yet the relevance of this finding was considered uncertain. Dr. Eustis said that uncertainty in interpretation is introduced because there is a transition point in the larynx from squamous to respiratory-type epithelium and it is difficult to get sections from precisely the same spot.

Dr. Davidson moved that the Technical Report of hexachlorocyclopentadiene be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, *no evidence of carcinogenic activity.* Dr. Bailey seconded the motion. Dr. Zeise offered an amendment that a sentence be added to the conclusions stating that rats might have been able to tolerate higher doses. Dr. Ward seconded the amendment, which was then defeated by two yes votes (Drs. Ward and Zeise) to eight no votes. The original motion by Dr. Davidson was then accepted unanimously with ten votes.